REMARKS/ARGUMENTS

Favorable reconsideration and continued examination of the present application are respectfully requested.

In the Office Action of July 8, 2005, claims 9, 13, 15 - 17, 20 - 27, 30, and 31 were rejected. No claim was allowed. In the present amendments to the claims of the application, claim 26 is canceled and claims 16, 20, 21, 24, 25, 27, and 30 are amended. Support for the amendments may be found throughout the specification and claims as originally filed, including, for example, page 9, lines 14 - 19 and page 19, lines 12 - 15 and in Fig. 8. Accordingly, no questions of new matter should arise, and entry of the amendment is respectfully requested.

Amendment to the Sequence Listing

The Sequence Listing is amended to correct an error that was made previously in the application and to restore SEQ ID NOs: 1-7 to their original and correct form.

Briefly, the application as originally filed on March 20, 2002, included a Sequence Listing that contained SEQ ID NOs: 1-7, which are the IGR-IRES regions of various CrP-like viruses. These sequences are referred to in the specification and claims. The specification also included drawing Figs. 1 and 2 that illustrate the alignment of the sequences and Fig. 4 that illustrates the conservation of higher order structure in the sequences. Each of the sequences illustrated in Figs. 1, 2, and 4 included the same base sequence set forth in the Sequence Listing, but also contained about 12 additional bases at the 3' end of each sequence representing the beginning portion of a virus coat protein encoding region.

On July 18, 2002, an amended Sequence Listing was filed. The amendment of July 18, 2002, erroneously replaced the sequences of SEQ ID NOs: 1-7 with the longer sequences illustrated in Figs. 1, 2, and 4, thereby inadvertently changing the meaning of the specification and claims that refer to SEQ ID NOs: 1-7.

In the present amendment, the Sequence Listing is amended so that SEQ ID NOs: 1-7 are the seven sequences that were given this designation in the original sequence listing. In order to maintain compliance with the sequence listing rules, the seven sequences from Figs. 1, 2, and 4 are re-designated as sequences SEQ ID NOs: 13-19. No sequences are removed from the Sequence Listing in the present amendment.

Since all of the described sequences were disclosed either in the original sequence listing or in the drawings, no questions of new matter should arise, and entry of the amendment is respectfully requested.

Objection to the Disclosure

At the bottom of page 3 of the Office Action, the Examiner objected to the disclosure because the description of the drawings at page 5 of the specification does not identify the sequences of Figs. 1, 2, and 4 with the proper "SEQ ID NO:" designation. In response, the specification is amended to identify the sequences in Figs. 1, 2 and 4 with respect to their proper "SEQ ID NO:" designations which, after the present amendment, are SEQ ID NOs: 13-19, respectively. Accordingly, the objection should be withdrawn.

Rejection of claims 9, 13, 15 - 17, 20, 21, 24, 26, 27, 30, and 31 under U.S.C. §112, first paragraph- written description

At page 4 of the Office Action, the Examiner rejected claims 9, 13, 15 - 17, 20, 21, 24, 26, 27, 30, and 31 under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the written description requirement. In the detailed comments on pages 4 - 8 of the Office Action, the Examiner took the position that while the specification provides a written description of the RNA higher-order structure that contains one of the sequences of SEQ ID NO: 1 - 7 or a sequence containing a mutation of PKI in the PSIV-IRES to permit translation of a GFP gene, the specification does not provide a written description of the genus of variants for an RNA higher-order structure with PK I, II, and III structures and a function of promoting translation activity, or a RNA higher-order structure made up of a base sequence having at least about 50% homology to the sequence of SEQ ID NO: 1 - 6 or 7, a complementary strand of the base sequence, a sequence hybridizing to the base sequence under stringent condition or a base sequence that has been mutated or altered. The Examiner further alleged that the specification does not provide sufficient teachings on the identities of the functional polynucleotides related to the sequences of SEQ ID NO: 1-7 and how to identify a functional polynucleotide among numerous polynucleotides related to the sequences of SEQ ID NO: 1-7. The Examiner alleged that a skilled artisan cannot envision all of the contemplated nucleotide sequences for an RNA higher-order structure based upon a specific RNA higher order structure of PSIV-IRES. For the following reasons, the rejection is respectfully traversed.

In one embodiment, the present invention relates to a method for synthesizing a heterologous protein in which the cell-free protein expressing system includes a polynucleotide

that promotes translation activity and a polynucleotide encoding a heterologous protein immediately downstream from the polynucleotide that promotes translation activity. The polynucleotide that promotes translation activity is further defined in the claims by having an RNA higher-order structure including PK (pseudoknot) I, II, and III structures. The present specification contains a thorough explanation of what is meant by an RNA higher-order structure including PK (pseudoknot) I, II, and III structures, for instance, in Example 1 at pages 14 - 15 and Figures 5 - 6 of the present specification. The genus of polynucleotides that contain the RNA higher-order structure including PK (pseudoknot) I, II, and III structures is thoroughly described, for instance, at pages 4 - 8 of the present specification. Accordingly, the Examiner is in error in alleging that the present specification does not disclose a genus of variants for the RNA higher order structure and in alleging that the subject matter is not described in the present specification in such a way as to reasonably convey to one skilled in the art that the inventors had possession of the claimed invention.

Further, with respect to the specific invention claimed herein, the Examiner is in error in asserting that the written description requirement requires a teaching of all of the possible nucleotide sequences for the RNA structures according to the present invention. In particular, the guidelines provided by the USPTO in the M.P.E.P, Section 2163 state that the written description requirement may be satisfied through a sufficient description of a representative number of species or by disclosure of relevant, identifying characteristics, such as structure or other physical or chemical properties. The present invention provides both: it provides a thorough description of the higher order structure including PK I, II, and III structures and it provides seven examples (SEQ ID NO: 1-7) of polynucleotides containing this structure, with a thorough illustration in

Figures 4 - 6 of how the higher order structure is formed by base pairing in each of SEQ ID NO: 1-7. Further, this section of the MPEP specifically states that the requirement of a disclosure of a representative number of species does not require individual support for every species that the genus embraces. Therefore, the Examiner is in error in alleging that the written description is not met based on the allegation that a skilled artisan cannot envision all of the contemplated nucleotide sequences. Clearly, the specification provides a sufficient teaching for a person skilled in the art to do so. Moreover, regarding the comments by the Examiner at page 7 of the Office Action that the specification does not teach how to identify a functional polypeptide among sequences related to SEQ ID NO: 1-7, the specification clearly teaches, for instance, at pages 14 - 15 that a polynucleotide that has the base pair and stem loop formation for forming the higher order structure for promoting translation can be determined with an RNA secondary structure-predicting program such as MFOLD, and the formation of a higher order structure can be readily identified from structures that have the correct stem loop formation in their secondary structure by determining base pairing as illustrated in Figures 5 and 6. Therefore, identifying functional polypeptides according to the present invention is well within the skill of persons skilled in the art combined with the teachings of the present specification.

Moreover, the Examiner has not presented any specific reason for including claim 24 in this rejection. Claim 24 is directed to the method of the present invention wherein the RNA higher-order structure comprises a base sequence selected from one of the sequences of SEQ ID NOs: 1-7, except that the base sequence contains an alteration in one or more combinations of base pairs that make up PKI so that polynucleotide that promotes translation activity is able to initiate translation activity of a heterologous protein or heterologous peptide without an AUG

translation initiation codon. Embodiments containing alterations in one or more combinations of base pairs that make up PKI and that allow for translation activity without an AUG translation initiation codon are clearly supported by the present application, for instance, at pages 13 - 14.

Accordingly, the present specification provides a written description of the claimed invention, and the rejection should be withdrawn.

Rejection of claims 13, 15, 21, 25, 26, 30, and 31 under U.S.C. §112, second paragraph-indefiniteness

At page 8 of the Office Action, the Examiner rejected claims 13, 15, 21, 25, 26, 30, and 31 under 35 U.S.C. §112, second paragraph, as allegedly being indefinite.

Regarding claims 13, 15, 21, 30, and 31, the Examiner alleged that these claims are indefinite because the use of the term "a base sequence hybridizing with the base sequences of 1) to 4) under stringent conditions" does not specify the conditions of hybridization and does not specify what sequence the hybridized nucleotide has. Although the Applicants believe that persons skilled in the art upon reading the present specification would clearly know what conditions are required for hybridization, to assist the Examiner, independent claims 21 and 30 are amended to provide the stringent conditions that are set forth at page 9, lines 15 - 19 of the present specification. Accordingly, this rejection should be withdrawn.

Regarding claim 25, the Examiner alleged that the claim is indefinite as to the phrase "positions 158-159 are gg instead of aa" for SEQ ID NO: 1," on the alleged grounds that SEQ ID NO: 1 has aa at positions 159-160 and not 158-159. In response, claim 25 is amended to specify that the altered positions are 159-160. Accordingly, this rejection should be withdrawn.

Regarding claim 26, the Examiner alleges that the claim is indefinite because it has the same scope as claim 20. In response, claim 26 is canceled and claim 27 is amended to depend from claim 20. Accordingly, this rejection should be withdrawn.

Rejection of claims 9, 13, 15, 16, 20 - 23, and 26 under 35 U.S.C. §102(b) as anticipated by Sasaki et al. (J. Virology)

At page 9 of the Office Action, the Examiner continued to reject claims 9, 13, 15, 16, 20 - 23, and 26 under 35 U.S.C. § 102(b) as being anticipated by Sasaki et al (J. Virology, 73, 1219-1226 (1999)). The Examiner alleged that Sasaki et al. (J. Virology) teaches AUG-unrelated translation initiation that is mediated by the IRES of PSIV *in vitro* and that Sasaki et al. (J. Virology) teaches that the LUC gene (a heterologous protein) was translated when fused downstream of $IRES_{6201}$ or $IRES_{6264}$ and that $IRES_{6264}$ contains SEQ ID NO: 1. Further, the Examiner alleged that $IRES_{6201}$ has at least 50% homology to SEQ ID NO: 1. The Examiner noted that Sasaki et al. (J. Virology) does not specifically indicate that the IRES sequences have an RNA higher order, but the Examiner took the position that the sequences would be expected to have at least the PK I, II or III structure, since $IRES_{6264}$ contains SEQ ID NO: 1. The Examiner further alleged that the references teaches that in the CAT-IRES-LUC series of constructs, LUC genes without an AUG initiation codon were ligated to PSIV sequences as shown in Fig. 5A, and that the LUC gene was efficiently translated when fused downstream of nt 6201, referring to pCAT-$IRES_{6201}$-LUC and pCAT-$IRES_{6264}$-LUC in Fig. 5B. The Examiner alleged that the description of translation of the LUC gene meets the criteria of the claimed method, since $IRES_{6264}$ of PSIV contains SEQ ID NO:1 and would therefore have PKI, PKII and PKIII

structures. For the following reasons, this rejection is traversed.

Sasaki et al. (J. Virology) describes only the translation of a protein that is a fusion protein, that is, a protein that contains at least some of both the native virus coat protein and luciferase. On the other hand, Claims 20 and 30 of the present invention, as amended herein, clarify that in the synthesis of a heterologous protein or polypeptide according to the present invention, the polynucleotide encoding the heterologous protein or heterologous polypeptide is immediately downstream from the PKI structure of the polynucleotide that promotes translation activity. As described above, a previous error in the sequence listing has been corrected so that SEQ ID NO: 1-7 do not include the virus coat protein encoding region of the sequences illustrated in Figs. 1, 2, and 4. Therefore, in the polynucleotide used in the method of the present invention, there is no sequence of polynucleotides encoding a virus coat protein between the polynucleotide that promotes translation activity and the polynucleotide encoding the heterologous protein. This allows for the heterologous protein alone to be synthesized instead of a fusion protein that contains a portion of a virus coat protein along with the heterologous protein. A method of synthesizing a heterologous protein using such a construct is neither taught nor suggested by Sasaki et al (J. Virology). In fact, Sasaki et al (J. Virology) teaches away from the present invention by providing data that suggests that it is not possible to synthesize a protein using IRES unless a portion of the virus coat protein is included in the synthesis. As shown in the lanes 2 and 3 in Fig. 5 of Sasaki et al. (J. Virology), the authors of that reference, which include one of the present inventors, were not able to achieve Luc protein synthesis via IRES when using $IRES_{6192}$-Luc and $IRES_{6195}$-Luc that was constructed using the restriction enzyme *Bam*HI to ligate IRES with luciferase gene. As stated in Applicants' previous response, it is believed that

that the nucleotide sequence immediately upstream of the capsid-coding region (i.e., nucleotides 6188-6192) interacts with a loop segment (i.e., nucleotides 6163-6167) in the stem-loop structure located 15-43 nt upstream of the 5' end of the capsid-coding region to form a pseudoknot structure which is essential for translation of the capsid protein (page 1513, claims 9, 13, 16, 20-23, and 26). The Examiner further alleged that Sasaki et al. (PNAS) describes that various mutations in the pseudoknot structure have also been tested on IRES-mediated translation (Fig. 2), e.g., 6163_{CAU}-6190_{AUG} and 6163_{CUA}-6190_{UAG}, where efficient translation occurred (Fig. 2B, lanes 9 and 15; page 1513, right column; page 1514, left column; claims 24, 30, and 31). The Examiner took the position that although Sasaki et al. (PNAS) does not specifically recite the nucleotide sequence of SEQ ID NO: 1, it is known that the IRES of PSIV contains SEQ ID NO: 1, therefore, the reference anticipates the claimed invention. For the following reasons, this rejection is traversed.

The present claims are directed to methods of synthesizing a heterologous protein or heterologous polypeptide initiated by a polypeptide that promotes translation activity and that has the specified higher order structure. Sasaki et al. (PNAS) only discloses studies relating to synthesizing a viral protein with its native translation initiator (the capsid protein gene of PSIV and its IRES). Accordingly, Sasaki et al. (PNAS) does not teach or suggest any method of synthesizing a heterologous protein or polypeptide and does not teach or suggest a step of providing a polynucleotide encoding the arbitrary heterologous protein or heterologous polypeptide and a polynucleotide that promotes translation activity, wherein the polynucleotide encoding the heterologous protein or heterologous polypeptide is immediately downstream from the PKI structure of the polynucleotide that promotes translation activity. Therefore, the rejection

the use of *Bam*HI inhibits formation of the IRES higher-order structure to make it impossible to translate downstream immediately from PKI. See also, page 15, lines 12 - 15 of the present specification. Therefore, Sasaki et al. (J. Virology) not only does not teach or suggest method of synthesizing a heterologous protein by providing that a polynucleotide encoding the heterologous protein or heterologous polypeptide is directly downstream from polynucleotide that promotes translation activity, as required by the present claims, the reference also does not teach or suggest providing that the polynucleotide that promotes translation activity includes the RNA higher-order structure including PK (pseudoknot) I, II, and III structures when the polynucleotide encoding the heterologous protein or peptide is directly downstream. For these reasons, the rejection should be withdrawn.

Rejection of claims 9, 13, 15, 16, 20 - 24, 26, 30, and 31 under 35 U.S.C. §102(b) as anticipated by Sasaki et al. (PNAS)

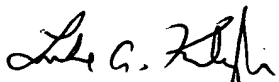
At page 12 of the Office Action, the Examiner rejected claims 9, 13, 15, 16, 20 - 24, 26, 30, and 31 under 35 U.S.C. § 102(b) as being anticipated by Sasaki et al. (PNAS 97, No. 4, 1512-1515 (February 2000). The Examiner alleged that Sasaki et al. (PNAS) teaches a group of positive-strained RNA viruses of insects that lack an AUG translation initiation codon for their capsid protein gene, and that the capsid protein of one of these viruses, *plautia stali* intestine virus (PSIV) is synthesized *in vitro* by internal ribosome entry side (IRES)-mediated translation (abstract, page 1513). The Examiner further alleged that Sasaki et al. (PNAS) describes that in the construct pT7CAT-5375 (Fig. 1), the translation of the capsid protein in this virus is initiated with glutamine encoded by a CAA codon that is the first codon of the capsid-coding region, and

should be withdrawn

Conclusion

If there are any fees due in connection with the filing of this response, please charge the fees to Deposit Account No. 50-0925. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such extension is requested and should also be charged to said Deposit Account.

Respectfully submitted,



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